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UTILITY PATENT APPLICATION TRANSMITTAL

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First Inventor or Application Identifier	Martin A. Cheever
Title	METHODS AND COMPOSITIONS TO GENERATE IMMUNITY IN HUMANS AGAINST SELF TUMOR ANTIGENS BY IMMUNIZATION WITH HOMOLOGOUS FOREIGN PROTEINS
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APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

ADDRESS TO: Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231

1. [ ] General Authorization Form & Fee Transmittal *(Submit an original and a duplicate for fee processing)*
2. [X] Specification [Total Pages] 24 *(preferred arrangement set forth below)*
   - Descriptive Title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) *(35 USC 113)* [Total Sheets] 17
4. Oath or Declaration [Total Pages]
   a. [ ] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 17 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b)
5. [ ] Incorporation By Reference *(useable if box 4b is checked)* The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. [ ] Microfiche Computer Program *(Appendix)*
7. Nucleotide and Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer-Readable Copy
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ACCOMPANYING APPLICATION PARTS

8. [ ] Assignment Papers (cover sheet & document(s))
9. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
10. [ ] English Translation Document *(if applicable)*
11. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
12. [ ] Preliminary Amendment
13. [X] Return Receipt Postcard
14. [ ] Small Entity Statement(s) [ ] Statement filed in prior application, Status still proper and desired
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[ ] Continuation [ ] Divisional [ ] Continuation-In-Part (CIP) of prior Application No : _____

Prior application information: Examiner _____ Group / Art Unit _____

[X] Claims the benefit of U.S Provisional Application No. 60/048,406

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METHODS AND COMPOSITIONS TO GENERATE IMMUNITY IN HUMANS AGAINST SELF TUMOR ANTIGENS BY IMMUNIZATION WITH HOMOLOGOUS FOREIGN PROTEINS

TECHNICAL FIELD

The present invention is generally directed toward generating immunity to self tumor antigens in humans. This invention is more particularly related to eliciting or enhancing immunity against human self tumor antigen by immunization with homologous foreign proteins.

BACKGROUND OF THE INVENTION

Despite enormous investments of financial and human resources, cancer remains one of the major causes of death. For example, cancer is the leading cause of death in women between the ages of 35 and 74. Standard approaches to treat cancer have centered around a combination of surgery, radiation and chemotherapy. Alternative approaches are needed not only to treat cancer, but also to prevent cancer.

A new generation of tumor antigens has been defined: "self proteins" (*J. Exp. Med. 180*:1-4, 1994; *Cell 82*:13-17, 1995). Self tumor antigens are proteins that are expressed by both normal cells and cancer cells. (As opposed to mutated proteins that are unique and thus cancer specific.) Self tumor antigens are typically overexpressed by the cancer cells. Certain self proteins, such as HER-2/*neu* and c-*myc*, are known to be involved in malignant transformation.

A common characteristic of malignancies is uncontrolled cell growth. Cancer cells appear to have undergone a process of transformation from the normal phenotype to a malignant phenotype capable of autonomous growth. Amplification and overexpression of somatic cell genes is considered to be a common primary event that results in the transformation of normal cells to malignant cells. The malignant phenotypic characteristics encoded by the oncogenic genes (oncogenes) are passed on during cell division to the progreny of the transformed cells.

Certain proto-oncogenes appear to be activated to a cellular oncogene through quantitative mechanisms that result from increased or deregulated expression

(overexpression) of an essentially normal gene product. For example, the *myc* gene family has been associated with initiation and/or progression of certain human lymphomas and carcinomas, whose transforming activation is the result of quantitative mechanisms. Proto-oncogenes are believed to be essential for certain aspects of normal cellular physiology. In this regard, the HER-2/*neu* oncogene is a member of the tyrosine protein kinase family of oncogenes and shares a high degree of homology with the epidermal growth factor receptor. HER-2/*neu* presumably plays a role in cell growth and/or differentiation. HER-2/*neu* appears to induce malignancies through quantitative mechanisms that result from increased or deregulated expression of an essentially normal gene product.

HER-2/*neu* (p185) is the protein product of the HER-2/*neu* oncogene. The HER-2/*neu* gene is amplified and the HER-2/*neu* protein is overexpressed in a variety of cancers including breast, ovarian, colon, lung and prostate cancer. HER-2/*neu* is related to malignant transformation. It is found in 50%-60% of ductal *in situ* carcinoma and 20%-40% of all breast cancers, as well as a substantial fraction of adenocarcinomas arising in the ovaries, prostate, colon and lung. HER-2/*neu* is intimately associated not only with the malignant phenotype, but also with the aggressiveness of the malignancy, being found in one-fourth of all invasive breast cancers. HER-2/*neu* overexpression is correlated with a poor prognosis in both breast and ovarian cancer. HER-2/*neu* is a transmembrane protein with a relative molecular mass of 185 kd that is approximately 1255 amino acids (aa) in length. It has an extracellular binding domain (ECD) of approximately 645 aa, with 40% homology to epidermal growth factor receptor (EGFR), a highly hydrophobic transmembrane anchor domain (TMD), and a carboxyterminal intracellular domain (ICD) of approximately 580 aa with 80% homology to EGFR.

Thus, HER-2/*neu* and c-*myc*, which are normal proteins found to be associated with malignant transformation when overexpressed, are examples of self tumor antigens. Other examples of self tumor proteins are those expressed by melanoma cells as melanocyte differentiation antigens, such as gp100, MAGE and MART-1. Self tumor proteins have been found to stimulate an immune response in

some patients whose cancers express those proteins (*e.g.*, *J. Exp. Med. 179*:921-930, 1994; *179*:1005-1009, 1994; and *180*:347-352, 1994). However, despite the presence of a detectable immune response to self tumor antigens in some patients, immunologic tolerance exists and represents a potential barrier to effectively vaccinating against tumor antigens. For example, rats vaccinated with either purified rat *neu* protein or rat *neu* extracellular domain (ECD) expressed by vaccinia virus do not develop rat *neu* specific immunity (*Proc. Natl. Acad. Sci. USA 84*:6854-6858, 1987). Tolerance can be circumvented in the rat, however, by immunization with peptides derived from the rat *neu* protein sequence. Nevertheless, the use of peptides may be problematic as they are thought of as weak immunogens and HLA restriction may limit usefulness (*e.g.*, by preventing the use of a single peptide for all patients). An ideal vaccine strategy targeting a self tumor antigen would be one in which vigorous immunity could be elicited with one vaccine formulation for all patients.

Due to the difficulties in the current approaches to treatment and prevention of cancer, there is a need in the art for improved methods and compositions. The present invention fulfills this need, and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides methods and compositions for eliciting or enhancing an immune response to a human self tumor antigen. The methods and compositions may be used on a one-time basis or on a periodic basis. The method comprises immunizing a human being with a foreign protein homologous to the antigen or with a foreign peptide homologous to a portion of the antigen.

In one embodiment of the method, the human self tumor antigen is a protein expression product of an overexpressed human oncogene. In a preferred embodiment, the antigen is human HER-2/*neu* protein. In another embodiment, the portion of the antigen is a portion of a protein expression product of an overexpressed human oncogene. In a preferred embodiment, the portion is a portion of human HER-2/*neu* protein. In a particularly preferred embodiment, the portion includes the intracellular domain of human HER-2/*neu* protein.

In another embodiment of the method, the human self tumor antigen or antigen portion is an organ-specific or tissue-specific differentiation antigen associated with tumor cells or a portion of the antigen. In a preferred embodiment, the human self tumor antigen or antigen portion is an antigen, or portion thereof, associated with prostate cancer. In a particularly preferred embodiment, the antigen is PAP. In another particularly preferred embodiment, the antigen is PSA.

In any embodiment of the invention, the foreign protein or foreign peptide is optionally in a pharmaceutically accepted carrier or diluent. Similarly, in any embodiment, an adjuvent may be additionally included.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-B show that rats immunized with intracellular domain of the human HER-2/*neu* protein (hICD) develop high titer human and rat *neu* specific antibodies. This figure represents data collected from 2 separate experiments with 8 experimental animals in each group. The greatest inter assay standard deviation, at the most concentrated control sera dilution, was 0.12 O.D. Three control animals that were not immunized are shown as an example of a naive rat response to human HER-2/*neu* and rat *neu* proteins. (A) Human HER-2/*neu* specific antibody responses were determined by ELISA. Results are depicted as the mean and standard deviation of the antibody response of each experimental group at each sera dilution. (B) Rat *neu* specific antibody responses were determined by ELISA. Results are depicted as the mean and standard deviation of the antibody response of each experimental group at each sera dilution.

Figure 2 shows that human HER-2/*neu* and rat *neu* specific antibodies, generated by immunizing with hICD, are specific for an intracellular domain epitope with 100% homology between rat and human *neu*. Sera derived from animals in each experimental group were evaluated in ELISA for antibody response to 16 peptides derived from the amino acid sequence of the rat *neu* protein structure. Eight of the

sixteen peptides were derived from sections of the rat *neu* protein that were 100% homologous with human *neu*. These peptides are underlined. This figure represents data collected from 2 separate experiments with 8 experimental animals in each group. Results are depicted as the mean and standard deviation of the antibody response of each experimental group at a sea dilution of 1:100.

Figure 3 shows that rats immunized with hICD develop DTH responses to rat *neu* protein. Animals were tested for DTH responses to rat *neu* protein 18-20 days after the last of two immunizations with hICD. The change in ear thickness is defined as the width of the experimental ear exposed to antigen in solvent minus the width of the control ear exposed to carrier solvent alone. Data is the mean and standard deviation of 4 animals in each experimental group.

Figures 4A-B show that immunization of rats with hICD elicits detectable T cell responses specific for both human and rat *neu* protein. (A) T cells ($1x10^5$) derived from draining lymph nodes of experimental rats were incubated with 1 μg/ml of recombinant hICD, purified rat *neu* protein or ova albumin as an irrelevant control protein. Proliferative responses were assayed after 4 days of culture in 6 well replicates. The data is expressed as a stimulation index which is the mean of the experimental wells divided by the mean of the control (no antigen) wells. Background proliferation of lymph node cells in media with no antigen ranged from 3275+/-790 to 9325+/-945 cpm. (B) T cells derived from spleens of immunized rats were assayed in the same fashion as the lymph node cells. Background proliferation ranged from 4795+/-725 to 8570+/-873 cpm. Data is the mean and standard deviation of 4 animals in each experimental group.

Figure 5 shows that rats immunized with hICD developed proliferative responses to both human and rat ICD protein in a dose dependent fashion. T cells ($2x10^5$) derived from spleens of immunized rats were incubated with increasing concentrations of recombinant hICD or recombinant rat ICD *neu* proteins. Proliferative responses were assayed after 4 days culture in 6 well replicates. The data is expressed in terms of a stimulation index which is the mean of the experimental wells divided by the mean of the control (no antigen) wells. Background proliferation of lymph node

cells in media with no antigen ranged from 835+/-84 to 11,584 +/-1450 cpm. None of the animals tested had an S.I. to ova albumin greater than 1.5 (data not shown). Data is expressed as the mean and standard deviation of 4 animals in each experimental group. This data represents a separate experiment from animals immunized in the experiment summarized in Figure 3.

Figure 6 shows that CTL lines generated by immunization with human HER-2/neu DNA recognized both naturally processed human HER-2/neu epitopes as well as murine HER-2/neu epitopes.

Figures 7A-B show that mice immunized with hICD develop antibodies (A) and T cells (B) specific for rat neu.

Figure 8 shows that animals immunized with hICD are protected against tumor challenge.

Figures 9A-B show Coomassie blue staining and western blot analysis of rat and human prostatic acid phosphatase (PAP). Rat (R) and human (H) PAP were run on a 10%-15% gradient SDS-PAGE gel under reducing conditions, followed by staining with Coomassie blue (A) or western blot analysis (B). The blot was incubated either with sera from female Lewis rat immunized with human PAP (*immune sera*) or with the polyclonal rabbit anti-rat PAP antibody as a positive control. In the second step, the blots were incubated with HRP-labeled goat anti-rat IgG (Caltag) or HRP-labeled donkey anti-rabbit Ig (Amersham), respectively. The blot was developed with the ECL (Amersham) detection system. The biotinylated molecular weight markers (M) (Biorad) are shown on the left.

Figures 10A-B show that no immunity to rat PAP was induced by immunization with whole rat PAP. Female Lewis rat were immunized with recombinant rat PAP (100 μg) admixed with CFA and boosted twice with rat PAP plus IFA at three-week intervals. The antibody response against rat PAP (rPAP) was determined by western blot analysis (A) as described in Figure 1. The T cell response was determined by a standard proliferation assay (B). Histapaque-purified splenic mononuclear cells ($5x10^5$/ml) were incubated with either media, rat PAP (200 μg/ml), or Con-A (5 μg/ml) for 96 hours. Tritiated thymidine (1 μCi/well) was added to the

culture for the last 8 hours. The thymidine uptake by T cells was determined by liquid scintillation counting (cpm).

Figure 11 shows the response to PAP-peptides and whole rat PAP in female rats. 12 week old Fischer (F344) rats were immunized (x3) with individual rat Prostatic Acid Phosphatase (PAP) peptides (100 μg) as well as whole rat PAP (100 μg), and then tested for a $CD4^+$ T cell response to both peptide and protein in a standard proliferation assay. The first immunization was Immunogen + CFA and the subsequent ones were in IFA. The analysis shown was done at the 4th *in vitro* stimulation.

Figure 12 shows that IgG antibody responses to rat PAP could be induced by sequential immunization with hPAP and rPAP. Female Lewis rats were immunized with human PAP (100 μg) plus CFA. They were boosted at three-week intervals with IFA plus human PAP (100 μg) and with IFA plus rat PAP (100 μg). Antibody responses to rat PAP or human PAP were determined by western blot analysis. Rat PAP (R) and human PAP (H) were run on a 10%-15% SDS-PAGE gel under reducing conditions. The blot was incubated with immune sera from two representative animals (2.1 and 2.2), followed by HRP-labeled goat anti-rat IgG antibody. The blot was developed with the ECL detection system. The molecular weight markers (M) are shown on the right.

Figure 13 shows that IgG antibody responses to rat PAP after immunization with human PAP can be significantly boosted by subsequent injections with rat PAP. Female Lewis rats were immunized with human PAP (100 μg) plus CFA. They were boosted sequentially at 3-week intervals with IFA plus human and rat PAP (hPAP x2, rPAP x1); or IFA plus human and rat PAP (hPAP x3, rPAP x2). Sera were obtained two weeks after each boosting. The amount of antibody to rat PAP (solid bars) or human PAP (open bars) in the immune sera was determined by an ELISA assay with 96-well plates pre-coated with either rat PAP or human PAP, respectively. The ELISA assay was developed with HRP-labeled goat anti-rat IgG antibody followed by HRP substrates, and the absorbance at 450 nm was determined.

Figure 14 shows that binding of IgG antibody in immune sera to immobilized rat and human PAP is inhibited significantly by soluble human PAP in an

ELISA assay. Sera (1:2000 dilution) from animals immunized with human PAP was pre-incubated with 100 ug/ml of either BSA, human PAP (hPAP) or rat PAP (rPAP) for 2 hours at 4°C. The sera was then added to 96-well plates pre-coated with either rPAP(100 μ/ml) or hPAP(100 μ/ml) to determine the amount of binding to rPAP (solid bars) or hPAP (open bars), respectively, by an ELISA assay. The ELISA assay was developed with HRP-labeled goat anti-rat IgG antibody followed by HRP substrates, and the absorbance at 450 nm was determined. The inhibition of antibody binding to immobilized rPAP and hPAP by pre-incubation of immune sera with either soluble rPAP or hPAP was compared to the binding of immune sera pre-incubated with BSA.

Figure 15A shows that both female and male rats can be immunized to rPAP by sequential immunization to foreign (human) PAP and rPAP. Female or male Lewis rats were immunized with human PAP and boosted with rat PAP as described in Figure 5. Sera obtained pre and post immunization was evaluated for a response to rat and human PAP by western blot analysis. Rat PAP (R) and human PAP (H) were run on a 10%-15% SDS-PAGE gel under reducing conditions. The blot was incubated with pre-immune or immune sera, followed by HRP-labeled goat anti-rat IgG antibody.

Figure 15B shows that similar IgG antibody responses to rat and human PAP were detected in male and female rats immunized with human PAP plus rat PAP, as determined by an ELISA assay. Female or male Lewis rats were immunized with human PAP and boosted with rat PAP as described in Figure 5. Sera obtained pre and post immunization was examined for antibody to rat and human PAP by an ELISA assay. The 96-well plates were first coated either with rat PAP (solid bars) or human PAP (open bars), and then incubated with pre-immune or immune sera (1:2000 dilution). The ELISA assay was developed with HRP-labeled goat anti-rat IgG antibody followed by HRP substrates, and the absorbance at 450 nm was determined.

Figure 16 shows that T cells from rats immunized sequentially to hPAP and rPAP can respond to rat PAP. Splenic T cells from rats immunized sequentially to hPAP and rPAP were stimulated once in vitro with rat PAP and then cultured with PBS or rat PAP (3, 30 and 300 μ/ml) in the presence of irradiated spleen cells as APC for 72

hours. One μCi of ^{3}H-TdR was added to the culture for the last 16 hours. Thymidine uptake was determined by liquid scintillation counting.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is directed toward methods and compositions to elicit or enhance immunity against human self tumor antigens. Because a self tumor antigen is a self protein (*i.e.*, a protein produced normally by an individual and not unique to a tumor), immunologic tolerance exists and represents a potential barrier to effectively vaccinating against such tumor antigens. The present invention overcomes immunologic tolerance by immunizing an individual with a protein or peptide that is foreign (*i.e.*, not identical to that in the individual) but nevertheless homologous to an individual's self tumor antigen or portion thereof.

As used herein, a "self tumor antigen" is a protein (including glycoproteins, lipoproteins, phosphoproteins, etc., or their amino acid sequences only) that is associated with a tumor in an individual, but has an amino acid sequence that is identical to a protein which is produced by the individual in the absence of the tumor. A variety of tumor antigens are self tumor antigens, and include the expression products of oncogenes. Certain oncogenes appear to induce malignancies through quantitative mechanisms that result from increased or deregulated expression of an essentially normal gene product. Self tumor antigens also include organ-specific and tissue-specific differentiation antigens associated with malignant cells. Self tumor antigens are associated with a variety of cancers including breast, ovarian, colon, lung and prostate.

Examples of self-tumor antigens that are the protein product of an oncogene that is amplified such that the protein is overexpressed by the tumor, include human HER-2/*neu* and the members of the *myc* family. Within the present invention, human HER-2/*neu* is a preferred self tumor antigen. An example of an organ that possesses organ-specific self tumor antigens is the prostate. Within the present invention, human prostate specific antigen (PSA) and human prostatic acid phosphatase (PAP) are preferred self tumor antigens.

The present invention discloses surprisingly that immunization of a human with a foreign protein that is homologous (*i.e.*, not identical) to a human self tumor antigen will result in the development of significant antibody and T cell responses to the self tumor antigens, including substantial reactivity to epitopes that are identical between the foreign protein and the self tumor antigen. As used herein, a "foreign protein homologous to a human self tumor antigen" means a protein that is homologous but not identical in entire amino acid sequence to a human self tumor antigen. Generally, a foreign protein will possess at least about 50% sequence homology to the self tumor antigen targeted. Sequence homology means either identical amino acids at the same positions in the sequence (*i.e.*, sequence identity), or conservative substitutions of amino acids at the same positions in the sequence. Conservative substitutions are well known in the art. Examples are isoleucine for leucine, valine for alanine, glutamic acid for aspartic acid, threonine for serine, etc. Typically, a foreign protein will possess about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence homology. Preferred foreign proteins are those which are highly homologous, *e.g.*, with 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater than 99% but less than 100% sequence homology. Particularly preferred foreign proteins are those wherein the aforementioned sequence homology percents each represent percent sequence identity.

Within the present invention, a foreign peptide may be used in place of, or in combination with, a foreign protein. Additionally, a foreign protein or peptide or both may be used in combination with a human self tumor antigen. Where two or more proteins/peptides are used in combination, they may be administered simultaneously or sequentially. As used herein, a "foreign peptide homologous to a portion of a human self tumor antigen" means a peptide that is homologous but not identical in its entire amino acid sequence to a portion of the amino acid sequence of a human self tumor antigen. The above discussion regarding sequence homology, percentages of sequence homology, preferred sequence homology percentages, preferred sequence identity percentages, etc., is applicable to foreign peptides and is incorporated by reference.

It will be evident to one of ordinary skill in the art that a foreign protein or foreign peptide for the present invention may be obtained in a variety of ways. For example a foreign protein or peptide may be purchased where available commercially. A foreign protein may be isolated from a non-human source. A foreign protein may be produced using a nucleic acid sequence encoding the foreign protein in combination with standard molecular biology methodologies. Alternatively, a foreign protein may be produced by standard molecular biology methodologies using a nucleic acid sequence which is a modified form of that encoding a self tumor antigen. For example, a nucleic acid sequence may be modified using random or site-specific mutagenesis. A foreign peptide may be similarly produced, or may be chemically synthesized (by manual or automated procedures) if its length is within the range appropriate for such methodologies. Automated peptide synthesizers are commercially available (*e.g.*, Perkin Elmer-Applied Biosystems Division, Foster City, CA). Commercial services are available to obtain peptides of a desired sequence (*e.g.*, Multiple Peptide Systems, San Diego, CA).

Another way to generate a foreign protein, or foreign peptide, for the present invention is to link a self tumor antigen, or portion thereof, to one or more essential differences in amino acid sequence between a self and a homologous foreign protein. An essential difference between amino acid sequences is a non-conservative substitution of an amino acid at the same position for the two sequences. Examples of essential differences are a charged versus a non-charged amino acid, an acidic versus a basic amino acid, etc. Such a foreign protein may be produced, for example, by fusion protein methodologies (nucleic acid sequences encoding desired portions of self and foreign proteins are combined) or site-specific mutagenesis (of nucleic acid sequence encoding self tumor protein to incorporate essential differences).

A variety of organisms produce proteins that qualify as foreign proteins. Such proteins (or peptides therefrom) may be used directly in the present invention or the information concerning their amino acid sequence (or sequence differences with human self tumor antigen) used to generate foreign proteins or foreign peptides. An example of an organism that produces foreign proteins homologous to human self

tumor antigens is a rat. For example, the rat *neu* protein is homologous but not identical in entire amino acid sequence of human HER-2/*neu* protein. Thus, the rat *neu* protein, or a peptide thereof, may be used as a foreign protein or peptide in the present invention to immunize a human being.

Immunization of an individual with a foreign protein or peptide (*e.g.*, as a vaccine) may include a pharmaceutically suitable carrier or diluent, such as physiological saline or sera. It will be recognized by one or ordinary skill in the art that the composition should be prepared in sterile form. Typically, about 0.01 μg/kg to about 100 mg/kg body weight will be administered by the intradermal, subcutaneous or intravenous route. A preferred dosage is about 1 μg/kg to about 1 mg/kg, with about 5 μg/kg to about 200 μg/kg particularly preferred. It will be evident to those skilled in the art that the number and frequency of administration will be dependent upon the response of the patient. It may be desirable to administer the foreign protein or peptide repetitively. It will be evident to one of ordinary skill in the art that more than one foreign protein or peptide may be administered, either simultaneously or sequentially.

In addition to the foreign protein or peptide (which functions as an antigen), it may be desirable to include other components with the foreign protein or peptide, such as a vehicle for antigen delivery and immunostimulatory substances designed to enhance the protein's immunogenicity. Examples of vehicles for antigen delivery include aluminum salts, water-in-oil emulsions, biodegradable oil vehicles, oil-in-water emulsions, biodegradable microcapsules, and liposomes. Examples of immunostimulatory substances (adjuvants) include N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), lipopoly-saccharides (LPS), glucan, IL-12, GM-CSF, gamma interferon and IL-15. When a peptide is used, it may be desirable to couple the peptide hapten to a carrier substance, such as keyhole limpet hemocyanin.

Immunization by the methods of the present invention results in the elicitation or enhancement of an immune response to a human self tumor antigen. Such an immunization may be performed for one of a variety of purposes. For example, it may be desired to elicit or enhance an immune response as a preventive measure to prevent tumor occurrence or recurrence, or as a therapy to arrest tumor growth or

eradicate existing tumors or to prolong the survival time. Eradicating tumor growth is based on stimulating an individual's immune system to induce a sustained destructive autoimmune response to cancer cells expressing the self tumor antigen. For example, eradicating prostate cancer cells is based on immunizing with a foreign protein or peptide to differentiation antigens expressed exclusively by the prostate gland in order to induce a sustained destructive autoimmune prostatitis. However, as described above, eliciting or enhancing an immune response to a human self tumor antigen can be useful even absent eradication of tumors.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1

HER-2/*NEU* IMMUNIZATION

A. Materials and Methods

1. *Animals:* Rats used in this study were Fischer strain 344 (CDF (F-344)/CrlBR) (Charles River Laboratories, Portage MI). Animals were maintained at the University of Washington Animal facilities under specific pathogen free conditions and routinely used for experimental studies between 3 and 4 months of age. Pathologic evaluation of rat tissues was performed by Dr. D. Liggitt, University of Washington, Department of Comparative Medicine.

2. *Neu Proteins.* Rat *neu* protein was purified using immunoaffinity column purification techniques. Briefly, a lysate preparation of a rat *neu* overexpressing cell line, DHFRG8 (ATCC, Rockville MD), was incubated overnight at 4°C on a prepared immunoaffinity Affigel-10 column (BioRad, Hercules, CA). $20x10^7$ cells were used to generate the lysate preparation (Disis et al., *J. Immunol. 156*:3151-3158, 1996). The Affigel-10 was coupled a rat *neu* specific antibody, 7.16.4 (kindly supplied by Dr. Mark Greene). After incubation with lysate, the column was washed three times, twice with PBS and once with 1 M NaCl. The rat *neu* protein was eluted with a buffer: pH 2.5, 0.05 M glycine, 0.15 M NaCl, and 0.1% Triton-X and the eluent

was immediately brought back to neutral pH with 1M Tris HCl. Pooled protein fractions were dialyzed against PBS. After dialysis, the protein was concentrated by centrifugation (Centricon-100, Amicon, Beverly, MA). The rat *neu* protein was sterile filtered (Nalgene, Rochester, NY). Protein purity was verified by both protein staining and Western blot (Disis et al., *ibid.*). Purified protein was quantified (Bio-Rad Protein Kit). Recombinant human and rat ICD proteins were kindly provided by Dr. Kennith Grabstein (Corixa Corp., Seattle, WA).

3. *Immunization:* Rats were immunized recombinant human HER-2/*neu* intracellular domain protein (hICD) (50 μg) , or immunoaffinity column purified rat *neu* protein (50 μg). Proteins were administered with either CFA (Sigma ImmunoChemicals, St. Louis, MO) or murine GM-CSF 5 μg (Immunex Corp., Seattle, WA) as adjuvants. Control groups received adjuvant alone. Inoculations with GM-CSF were given id and inoculations with CFA were administered sq. Animals underwent 2 immunizations each 14-16 days apart. 18-20 days after the second immunization animals were assessed for immunologic response. DTH responses to rat *neu* protein were assessed. Sera, spleens and draining lymph nodes were harvested from immunized animals. Experiments included 4 animals/experimental group. Data shown here was derived from two separate immunization experiments for each group performed more than 2 months apart.

4. *Cell Lines:* Two cell lines were used as a source of *neu* proteins. SKBR3, a human breast cancer cell line that is a marked overexpressor of HER-2/*neu* (American Type Culture Collection, Rockville, MD), was maintained in culture in 10% fetal bovine serum (FBS) (Gemini Bioproducts, Inc., Calabasas, CA) and RPMI. DHFRG8, an NIH/3T3 cell line cotransfected with c-*neu*-p and pSV2-DHFR (American Type Culture Collection, Rockville, MD), was used as a source of non-transforming rat *neu* protein. This cell line was maintained in 10% FBS and Dulbecco's modified Eagle's medium with 4.5g/L glucose. DHFRG8 cells were passaged through the same medium supplemented with 0.3 μM methotrexate at every third passage to maintain the *neu* transfectant.

5. *ELISA for rat neu and human HER-2/neu specific antibody responses:* 96 well Immulon 4 plates (Baxter SP, Redmond, WA: Dynatech Laboratories) were incubated overnight at 4°C with either a rat *neu* specific antibody (c-*neu*-4, Oncogene Science) or a human HER-2/*neu* specific antibody (520-C9, a kind gift of Dr. David Ring) at a concentration of 10 μg antibody per ml in carbonate buffer. After incubation, all wells were blocked with PBS and 1% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO), 100 μl/well for 4 hours at room temperature. The plate was washed with PBS/0.5% Tween and protein was added. Rows of wells were coated with alternating PBS/1%BSA and DHFR-G8 lysate (rat *neu*) or SKBR3 lysate (human HER-2/*neu*) (108 cells/20 ml PBS), 50 μl/well, overnight at 4°C. After washing, the plate was incubated with rat sera at the varying dilutions in PBS/1% BSA and incubated 1 hour at room temperature. Sheep anti-rat Ig horseradish peroxidase (HRP) was added to the wells at a 1:5000 dilution in PBS/1%BSA and incubated for 45 minutes at room temperature (Amersham Co.). Following the final wash, TMB (Kirkegaard and Perry Laboratories, Gaithersburg, MD) developing reagent was added. The optical density was read at 450 nm. The OD of each serum dilution was calculated as the OD of the *neu* coated wells minus the OD of the PBS/1%BSA coated wells.

Antigen specificity was confirmed by analyzing experimental sera for antibody responses to ova albumin in an ELISA. In these analysis, plates were incubated overnight at 4°C with purified ova albumin protein at 10 μg/ml concentration in carbonate buffer alternating with rows of buffer alone. Antibody evaluation proceeded as described above.

6. *ELISA for peptide epitope analysis:* 96 well Immulon 4 plates (Dynatech Laboratories) were incubated overnight at 4°C with *neu* peptides at a concentration of 10 μg/well diluted in PBS alternating with rows of PBS/1%BSA. The peptides constructed were 15-18 amino acids in length and were derived from the amino acid sequence of the rat *neu* protein. Some peptides were located in areas of 100% homology between rat *neu* and human HER-2/*neu*. The peptide coated plate was incubated with rat sera diluted 1:50 and 1:100 for 1 hour at room temperature. Sheep

anti-rat HRP was added to the wells at a 1:5000 dilution in PBS/1%BSA and incubated for 45 minutes at room temperature. Following the final wash, the TMB developing reagent was added. The optical density was read at 450 nm. The OD of each serum dilution was calculated as the OD of the peptide coated wells minus the OD of the PBS/1%BSA coated wells.

7. *Delayed Type Hypersensitivity (DTH) responses*: 18 days after the final inoculation baseline ear thickness was measured in each animal using a dual thickness gauge (Mitutoyo Corporation, Japan). Immediately following the baseline measurement, the left ear was treated epicutaneously with a carrier solvent consisting of a 1:1 mix of acetone and dibutyl pthalate. 10 µl of the carrier solvent was applied to the front of the ear and 10 µl was applied to the back of the ear. The right ear of each animal was treated with the carrier solvent and antigen with 10 µl of the carrier diluted antigen mix applied to the front of the ear and 10 µl applied to the back of the ear. Animals were tested with 1 µg/ml of purified rat *neu* protein. DTH response as a measure of ear thickness was measured at 48 hours and calculated as the difference in the thickness of the experimental ear compared to control.

8. *T cell proliferation assays:* For analysis of *neu* protein specific responses, immune spleen cells were harvested by mechanical disruption and passage through wire mesh and washed. $2x10^5$ spleen or $1x10^5$ lymph node cells/well were plated into 96-well round bottom microtiter plates (Corning, Corning, NY) with 6 replicates per experimental group. The media used consisted of EHAA 120 (Biofluids) with L-glutamine, penicillin/streptomycin, 2-mercaptoethanol, and 5% FBS. In initial experiments, cells were incubated with 1 µg/ml of the various proteins. Subsequent experiments evaluated increasing concentrations of experimental proteins, recombinant human HER-2/*neu* ICD and recombinant rat *neu* ICD, ranging from 0.5 to 2.0 µg/ml. After 4 days, wells were pulsed with 1 µCi of [3H]thymidine for 6-8 hours and counted. Data is expressed as a stimulation index which is defined as the mean of the experimental wells divided by the mean of the control wells (no antigen). Ova albumin was used as a negative control antigen for proliferation in all assays at a 1 µg/ml concentration.

B. Results

1. *Rats immunized with hICD develop high titer human and rat neu specific antibodies.* Previous studies demonstrated that rats, immunized with rat *neu* protein, do not develop immune responses to rat *neu* (Bernards et al., *Proc. Natl. Acad. Sci. USA 84*:6854-6858, 1987). Animals are presumed tolerant to this "self" protein. For the current study, rats were given a priming immunization and a boost immunization with hICD with either GM-CSF or CFA as an adjuvant. All rats immunized with hICD developed significant antibody responses specific for human HER-2/*neu* protein, with titers greater than 1:200,000 (Figure 1A). By marked contrast, rats immunized with rat *neu* protein did not develop human *neu* specific antibodies. Ova albumin was used as a negative control protein. No sera tested was positive for antibodies to ova.

Human HER-2/*neu* ICD is 92% homologous to rat *neu* ICD at the amino acid level. Analysis was performed to discern whether the human HER-2/*neu* specific antibodies were cross-reactive with rat *neu*. Rats immunized with hICD with either GM-CSF or CFA as an adjuvant had high titer antibody responses specific for rat *neu* (Figure 1B). The magnitude of the rat *neu* specific antibody responses was nearly identical to that of the human HER-2/*neu* specific response.

Human HER-2/*neu* and rat *neu* specific antibodies, generated by immunizing with hICD, are specific for an intracellular domain epitope with 100% homology between rat and human *neu*. Epitope mapping was done with a series of synthetic peptides (n=16) derived from the amino acid sequence of the rat *neu* protein. Both intracellular and extracellular peptides were included. Eight of the peptides were derived from region of the rat *neu* protein that were 100% homologous with human HER-2/*neu* protein. The dominant response detected was to an ICD peptide epitope, p932-946 (Figure 2). The amino acid sequence of this peptide is identical between rat and human.

2. *Rats immunized with hICD develop DTH responses to rat neu protein.* The conditions for circumventing T cell tolerance may be more stringent than those needed to break B cell tolerance. Key for a successful cancer vaccine targeting a "self" tumor antigen is the ability to generate significant T cell immunity. DTH

responses were used to initially evaluate for the presence of the T cell responses to *neu* in rats immunized with HER-2/*neu*. HER-2/*neu* specific DTH responses were detected in animals who received hICD in GM-CSF or CFA (Figure 3). The responses were cross-reactive to rat *neu* protein. DTH was not detected in animals immunized with rat *neu* protein or with adjuvants alone.

3. *Immunization of rats with hICD elicits detectable T cell responses specific for both human and rat neu protein.* T cell proliferative responses were evaluated in rats immunized with hICD plus either GM-CSF or CFA. T cell responses to hICD protein were detected from lymph nodes draining the inoculation site (Figure 4A) and spleen (Figure 4B). T cell responses to rat *neu* protein were also detected, although at a lower magnitude than the hICD response. There was no evidence of response to an irrelevant protein, ova albumin. Animals immunized with rat *neu* protein with adjuvants or adjuvants alone did not have a detectable T cell response to either hICD or rat *neu* protein.

Rats immunized with hICD developed significant proliferative responses to both human and rat ICD protein in a dose dependent fashion (Figure 5). The magnitude of the T cell immune responses directed against rat or human *neu* protein was similar in rats immunized with hICD plus CFA at the greatest concentration of antigen tested (2.0 μg). The magnitude of the T cell response against rat was less than the response to human in rats immunized with hICD plus GM-CSF at all concentrations. However, the possibility exists that the responses would become more equivalent with additional boosting.

Biopsies of skin, liver, lung, gastrointestinal tract, kidney and heart were obtained from immunized animals and evaluated for histopathologic evidence of autoimmunity. There was no evidence of autoimmune pathology in these tissues which express basal levels of rat *neu* protein.

4. *CTL lines generated by immunization with human HER-2/neu DNA recognized both naturally processed human HER-2/neu epitopes as well as murine HER-2/neu epitopes.* BALB/c mice were immunized with human HER-2/neu DNA (100Ug x3 id.). Spleen cells were cultured for 5 days with WEHI cell line

transfected with HER-2/neu as stimulator cells. CTL activity was assessed in a standard 4 hour chromium release assay on day 5. The targets included WEHI alone, WEHI transfected with HER-2/neu, WEHI incubated with the HER-2/neu intracellular domain (ICD) peptide denoted p780-788 (identical in humans and mice) or with WEHI incubated with the HER-2/neu murine extracellular domain peptide denoted p63-71.

5. *Mice immunized with hICD develop antibodies and T cells specific for rat neu.* Figures 7A-B show the generation of immunity in another self model—the neu transgenic mouse. This is a mouse which has rat neu on an MMTV promoter and develops breast cancer mediated by overexpression of rat neu in mid to late life. In this mouse, rat neu is a self protein. Figure 7A shows that if this mouse is immunized with human ICD, a rat neu antibody response is obtained. Figure 7B, the T cell response, shows significant rat and human T cell immunity.

6. *Immunization of animals with hICD protein vaccine protects against tumor challenge.* Figure 8 demonstrates that these immune responses are protective. Animals who received two immunizations with human ICD, a foreign highly homologous protein, prior to tumor implant were totally protected from tumor development as compared to animals who received adjuvant alone.

EXAMPLE 2

PAP IMMUNIZATION

A. Materials and Methods

1. *Recombinant rat and human PAP were expressed and purified.* Recombinant rPAP was obtained from Dr. P. Vihko. rPAP was expressed in baculovirus and purified as described previously. rPAP runs as a 40 kD protein under reducing conditions on a 10-15% SDS gel (Figure 9A). The protein readily forms dimers when not completely reduced. Recombinant hPAP for use as a homologous foreign protein in the immunization studies was also obtained from Dr. Vihko. Human PAP, expressed in baculovirus, runs as a 45kD protein under reducing conditions (Figure 9A) and also has a propensity to form dimers. Polyclonal rabbit anti-rPAP, elicited by immunization with a 15 aa long C-terminal peptide derived from rPAP was

specific for recombinant rPAP and did not cross-react with hPAP (Figure 9B). Polyclonal rat anti-hPAP, elicited by immunization of female Lewis rats with hPAP, cross reacted with human and rPAP (Figure 9B).

B. Results

1. *No immunity to rPAP is induced by immunization with native rat PAP protein.* Initial experiments tested immunization to PAP in female rats with the presumption that female rats express substantially lower levels of PAP than male rats. This presumption was an extrapolation from human studies in which PAP is not detectable in females. Although female rats are not known to express PAP, some domains of PAP are shared with other phosphatases. Thus, portions of PAP are known to be expressed in females but whether females are tolerant to the whole PAP protein was unknown. Female rats were injected sc. with 100 ug of recombinant rPAP with complete Freund's adjuvant (CFA) and boosted twice with rPAP in incomplete Freund's adjuvant (IFA). No antibody (Figure 10A) or T cell response to rPAP could be detected (Figure 10B). In many of the subsequent experiments, serum antibody (IgG) to PAP is used as a surrogate read out for helper T cell immunity.

2. *IgG antibody responses to rat PAP can be induced in female rats by concurrent immunization with human PAP and rat PAP.* In initial attempts to test for immunization to PAP, immunization with the combination of bovine PAP and rPAP was tested. Female rats were immunized with 100 ug of bovine PAP in CFA x 1, followed by 100 ug of rPAP in IFA x 2. There was no antibody response to bovine PAP, and no detectable antibody response to rPAP.

Subsequent experiments tested immunization with the combination of hPAP and rPAP. Female Lewis rats were immunized with 100 μg hPAP x 2, followed by 100 μg rPAP x 1. Strong IgG antibody responses to both hPAP and rPAP were elicited (Figure 12). As in prior experiments, there was no antibody response to PAP in rats immunized with rPAP alone. Results were reproducible. In two separate experiments, 4/4 and 3/3 rats exhibited a strong antibody responses to both the human and rat proteins.

Antibodies to rPAP were reactive primarily to cross-reactive epitopes present on both rPAP and hPAP. Immunization to rPAP alone elicited no antibody (Figure 10). Immunization to hPAP elicited antibody primarily to hPAP, with a lower response to rPAP (Figure 13). Immunization to hPAP followed by immunization to rPAP boosted the response to rPAP to a level comparable to the response to hPAP (Figure 13). Despite the need for immunization with rPAP to elicit the highest level of antibody to rPAP, the epitopes recognized were present on both rPAP and hPAP. This was determined in experiments showing that antibody to rPAP could be absorbed out by incubation with hPAP, but antibody to hPAP could not be absorbed out by incubation with rPAP. In those experiments (Figure 14) pre-incubation of sera from rats immunized to hPAP plus rPAP (1:2000 dilution), hPAP (100 μg) in the solution phase competed out antibody to both hPAP and rPAP. By contrast, pre-incubation of immune sera with rPAP, successfully competed out only the anti-rPAP response, and did not appreciably effect the anti-hPAP response (Figure 14).

3. *Antibody and T cell responses to rat PAP can be induced in male rats by sequential immunization with human PAP and rat PAP.* In the above experiments, there was a much stronger anti-rPAP antibody response in female rats when animals are immunized sequentially with hPAP plus rPAP, as opposed to concurrent immunization with hPAP plus rPAP. Male rats were immunized with the optimal regimen for immunizing female rats above, *i.e.*, hPAP (100 μg) in CFA x 1 and boosted x 1 with hPAP and x1 with rPAP in IFA. Male rats developed a robust IgG antibody response to rPAP (Figure 15A), with the absolute level of response being very comparable to that elicited in female rats in the same experiment (Figure 15B). Importantly, T cells specific for rPAP were also elicited (Figure 16).

From the foregoing, it will be evident that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

CLAIMS

1. A method of eliciting or enhancing an immune response to a human self tumor antigen, comprising immunizing a human being with a foreign protein homologous to said antigen or with a foreign peptide homologous to a portion of said antigen.

2. The method of claim 1 wherein said antigen is a protein expression product of an overexpressed human oncogene.

3. The method of claim 2 wherein said antigen is human HER-2/*neu* protein.

4. The method of claim 1 wherein said portion of said antigen is a portion of a protein expression product of an overexpressed human oncogene.

5. The method of claim 4 wherein said portion is a portion of human HER-2/*neu* protein.

6. The method of claim 5 wherein said portion includes the intracellular domain of human HER-2/*neu* protein.

7. The method of claim 1 wherein said antigen or said antigen portion is an organ-specific or tissue-specific differentiation antigen associated with tumor cells or a portion of the antigen.

8. The method of claim 7 wherein said antigen or said antigen portion is an antigen associated with prostate cancer or a portion of the antigen.

9. The method of claim 8 wherein said antigen is PAP.

10. The method of claim 8 wherein said antigen is PSA.

11. The method of any one of claims 1-10 wherein said foreign protein or foreign peptide is in a pharmaceutically acceptable carrier or diluent.

12. The method of any one of claims 1-10 wherein an adjuvant is additionally used.

METHODS AND COMPOSITIONS TO GENERATE IMMUNITY IN HUMANS AGAINST SELF TUMOR ANTIGENS BY IMMUNIZATION WITH HOMOLOGOUS FOREIGN PROTEINS

ABSTRACT OF THE DISCLOSURE

Methods and compositions to elicit or enhance immunity in humans against self tumor antigens are disclosed. Such immunity is generated by immunization with homologous foreign proteins. Self tumor antigens include protein expression products of overexpressed human oncogenes, such as human HER-2/*neu* protein, and organ-specific or tissue-specific differentiation antigens, such as PAP or PSA, associated with tumor cells.

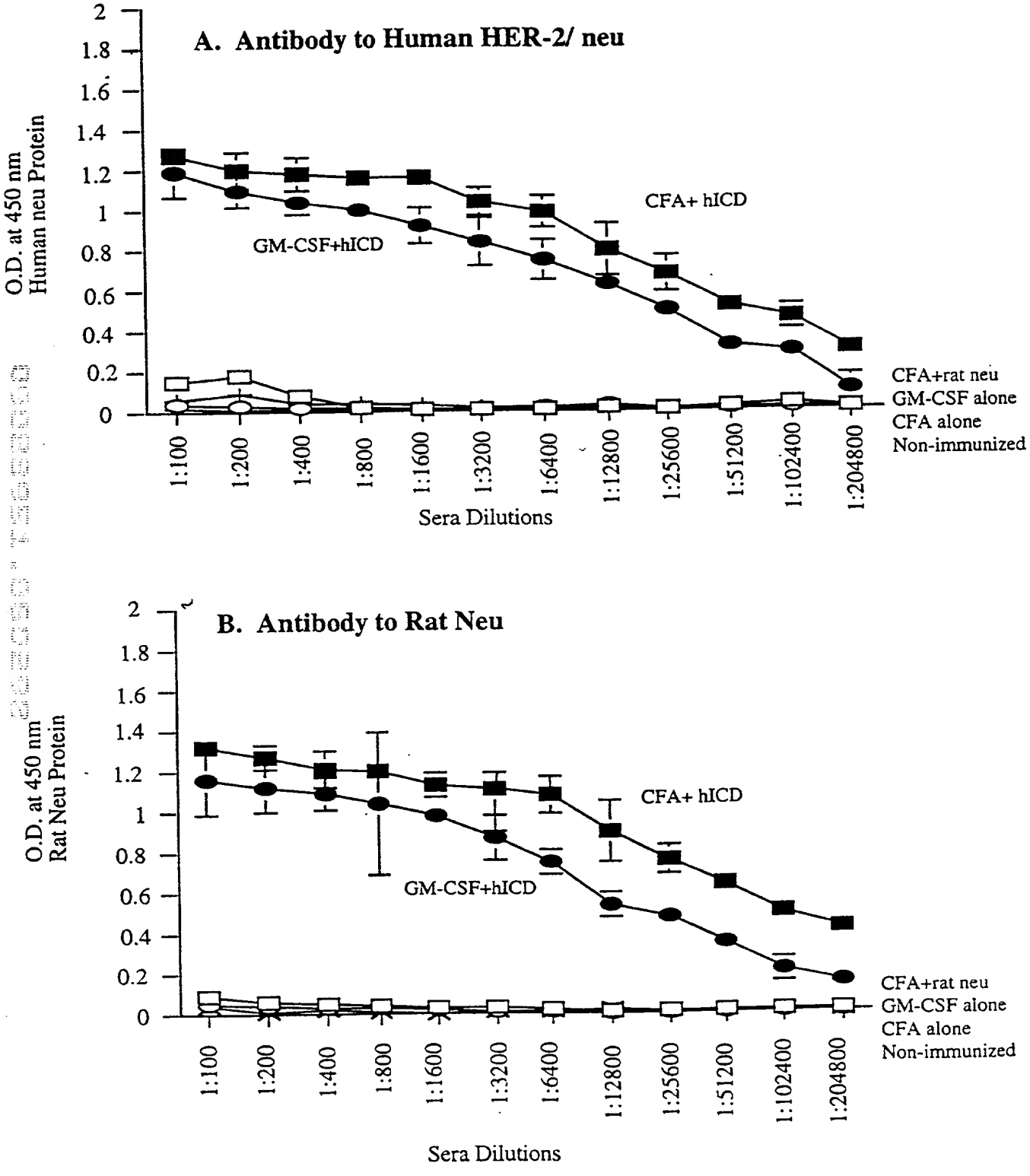

A. Antibody to Human HER-2/ neu
O.D. at 450 nm
Human neu Protein
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CFA+ hICD
GM-CSF+hICD
CFA+rat neu
GM-CSF alone
CFA alone
Non-immunized
1:100
1:200
1:400
1:800
1:1600
1:3200
1:6400
1:12800
1:25600
1:51200
1:102400
1:204800
Sera Dilutions
B. Antibody to Rat Neu
O.D. at 450 nm
Rat Neu Protein
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CFA+ hICD
GM-CSF+hICD
CFA+rat neu
GM-CSF alone
CFA alone
Non-immunized
1:100
1:200
1:400
1:800
1:1600
1:3200
1:6400
1:12800
1:25600
1:51200
1:102400
1:204800
Sera Dilutions

Figure 1

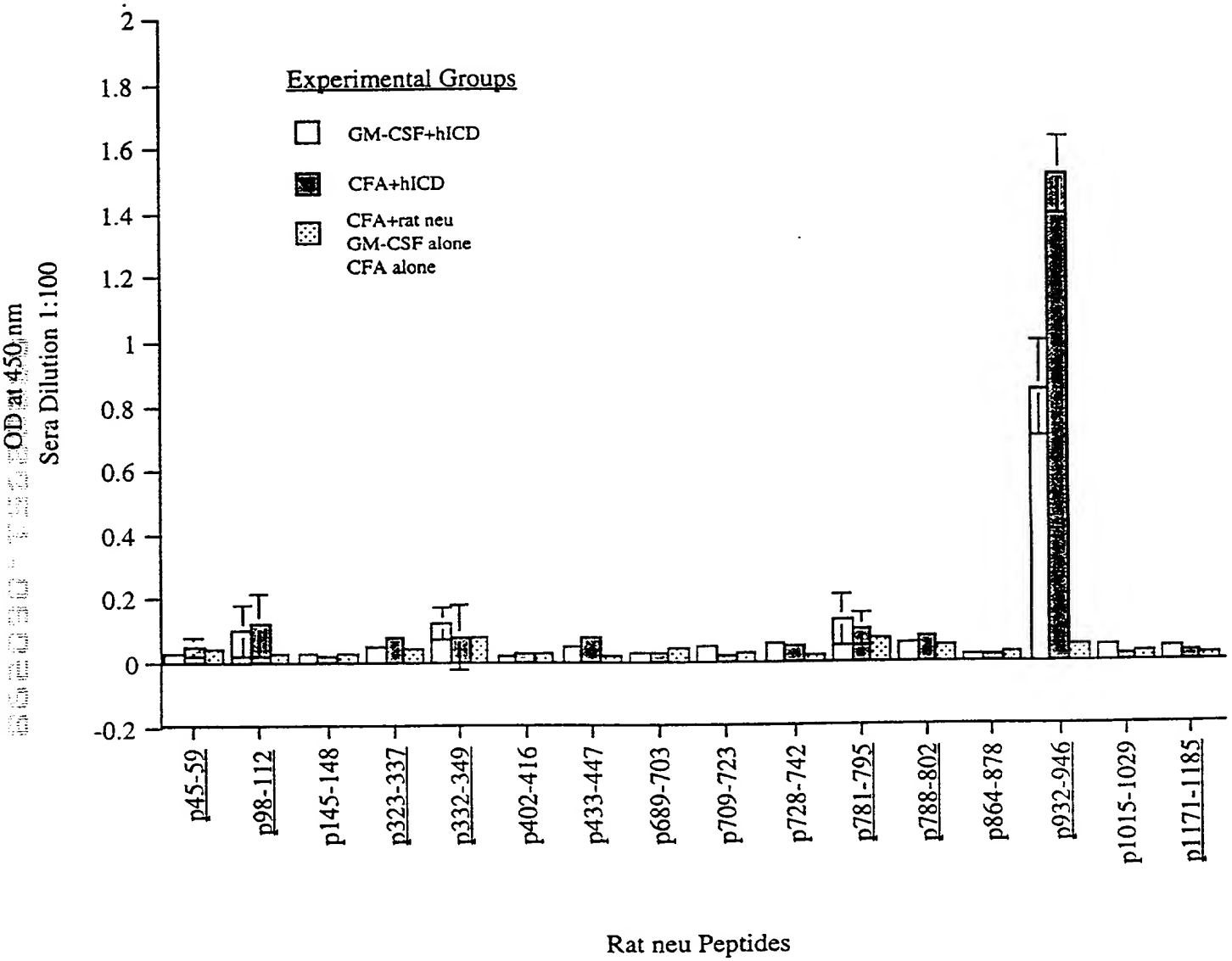

Experimental Groups
GM-CSF+hICD
CFA+hICD
CFA+rat neu
GM-CSF alone
CFA alone
Sera Dilution 1:100
OD at 450 nm
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
p45-59
p98-112
p145-148
p323-337
p332-349
p402-416
p433-447
p689-703
p709-723
p728-742
p781-795
p788-802
p864-878
p932-946
p1015-1029
p1171-1185
Rat neu Peptides

Figure 2

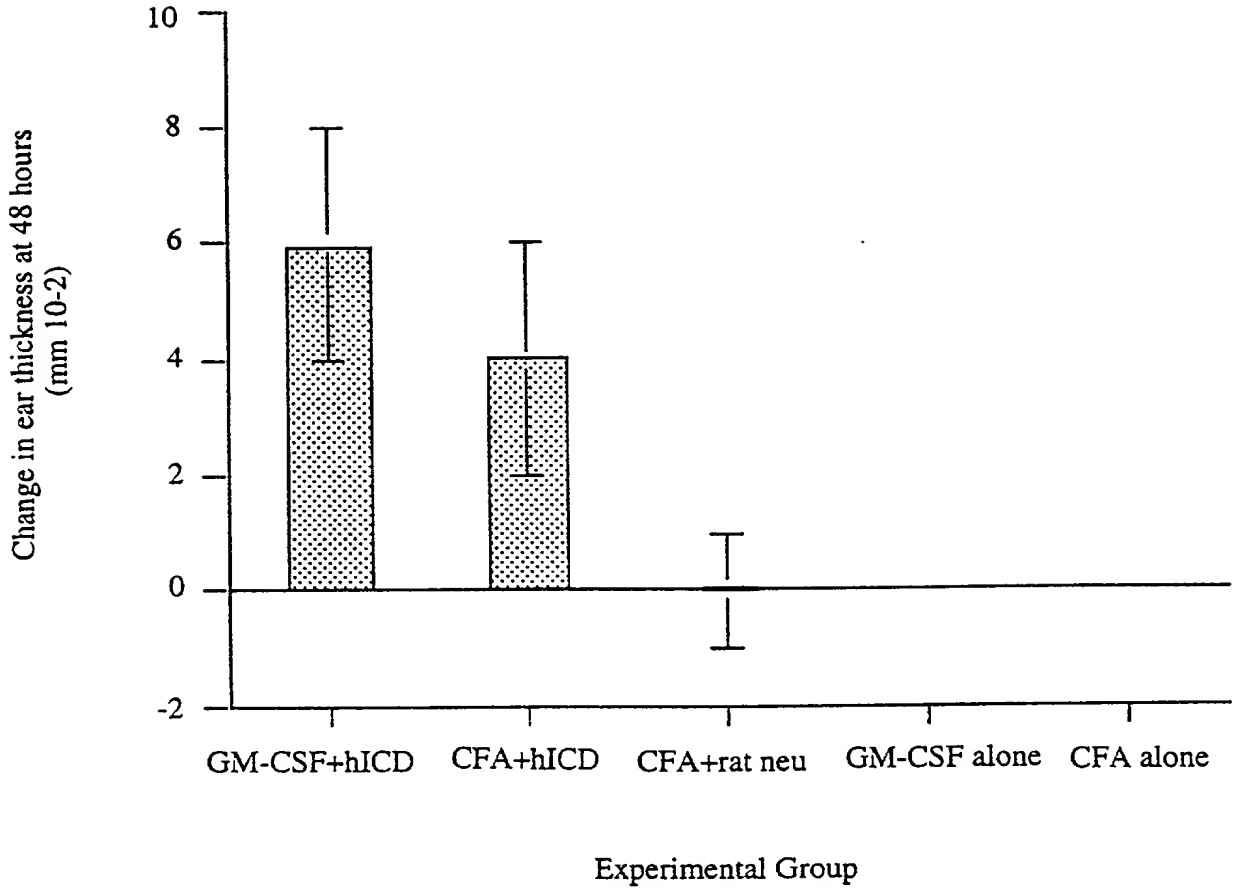
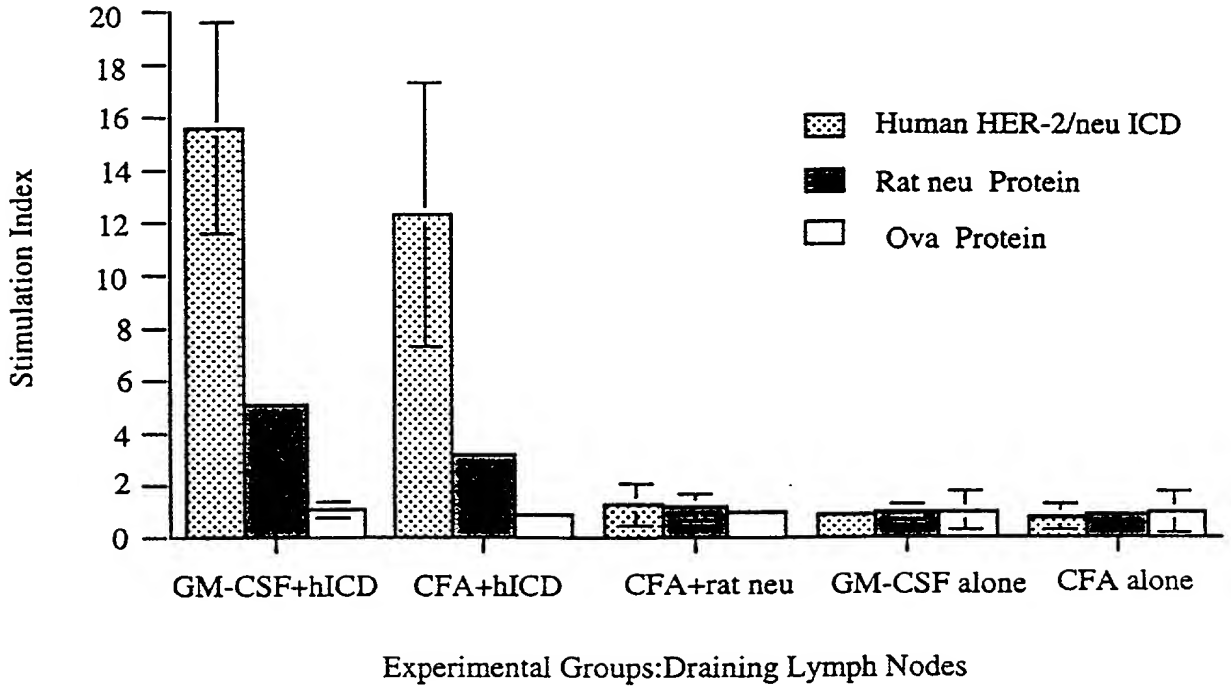

Change in ear thickness at 48 hours
(mm 10-2)
10
8
6
4
2
0
-2
GM-CSF+hICD
CFA+hICD
CFA+rat neu
GM-CSF alone
CFA alone
Experimental Group

Figure 3

A.


Stimulation Index
20
18
16
14
12
10
8
6
4
2
0
Human HER-2/neu ICD
Rat neu Protein
Ova Protein
GM-CSF+hICD
CFA+hICD
CFA+rat neu
GM-CSF alone
CFA alone
Experimental Groups:Draining Lymph Nodes

B.

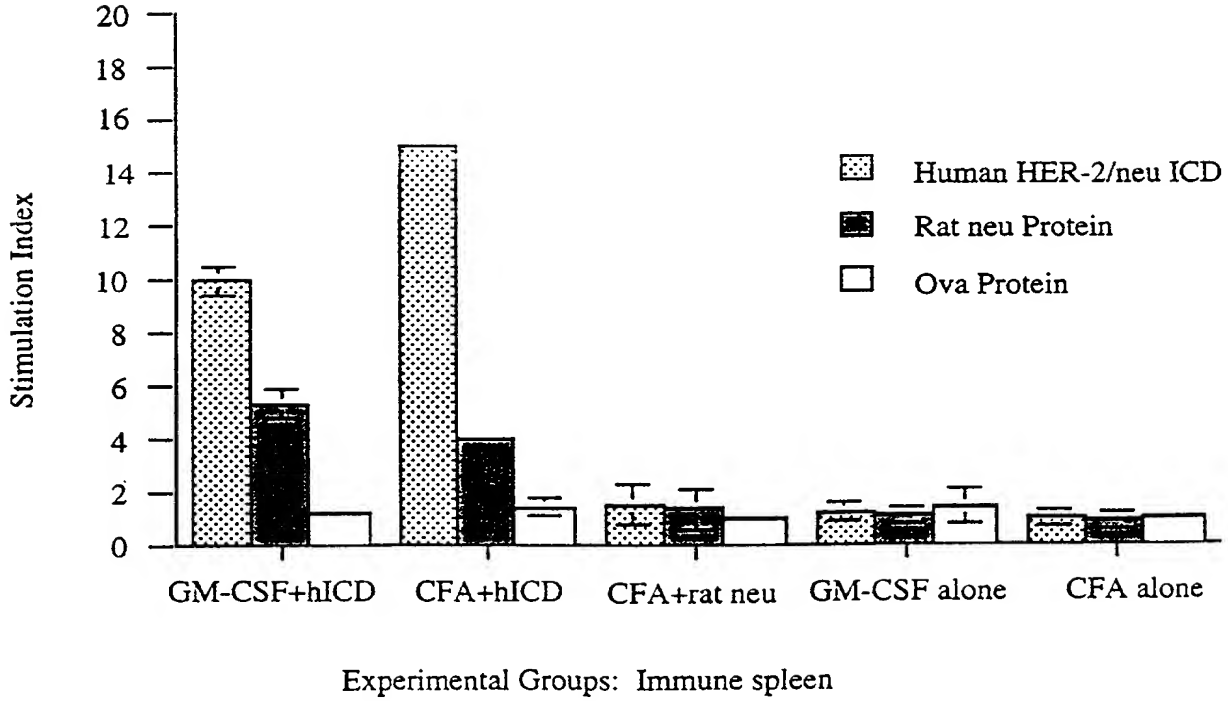

Stimulation Index
20
18
16
14
12
10
8
6
4
2
0
Human HER-2/neu ICD
Rat neu Protein
Ova Protein
GM-CSF+hICD
CFA+hICD
CFA+rat neu
GM-CSF alone
CFA alone
Experimental Groups: Immune spleen

Figure 4

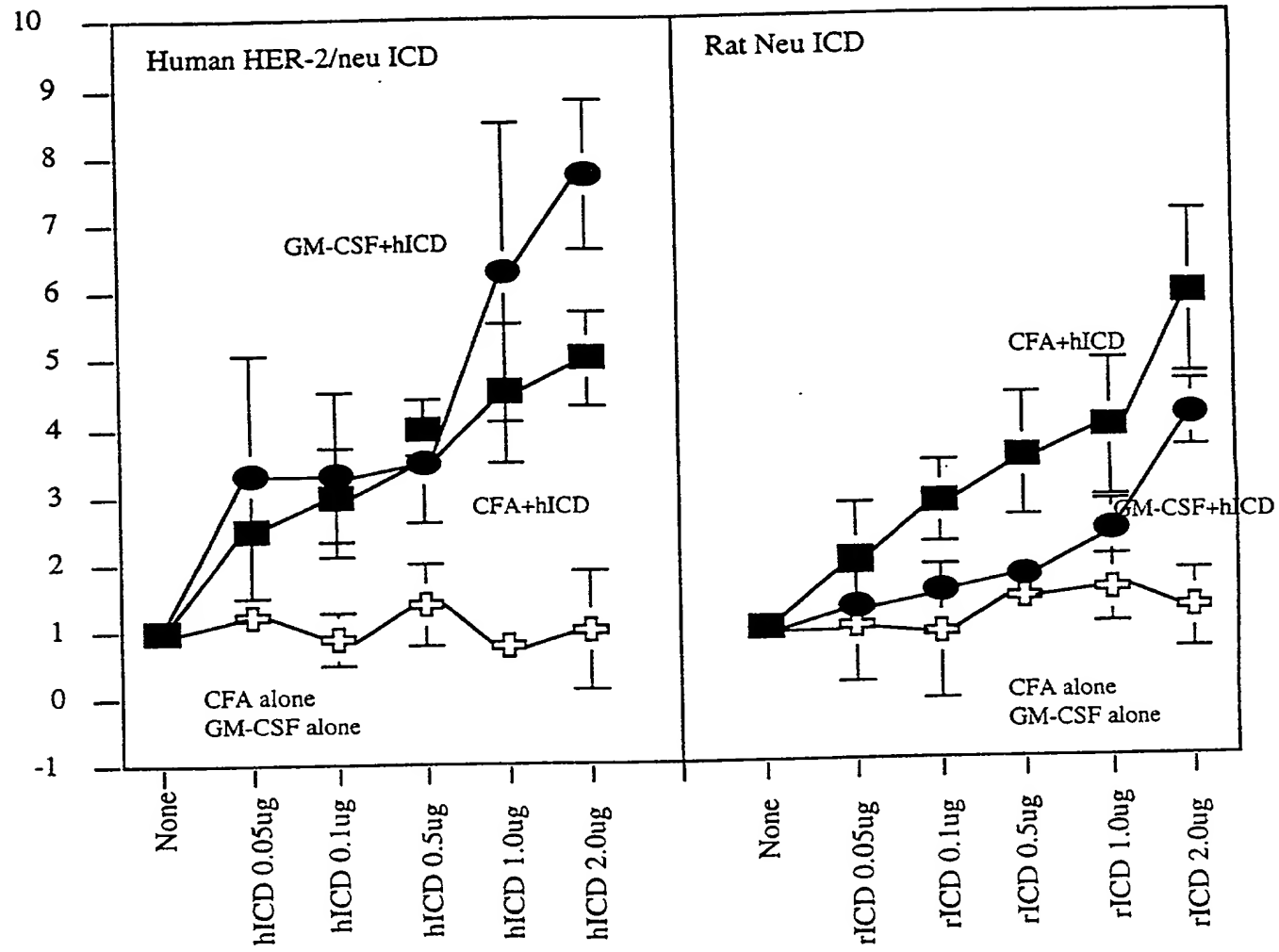

Human HER-2/neu ICD
Rat Neu ICD
Stimulation Index
10
9
8
7
6
5
4
3
2
1
0
-1
GM-CSF+hICD
CFA+hICD
CFA alone
GM-CSF alone
CFA+hICD
GM-CSF+hICD
CFA alone
GM-CSF alone
None
hICD 0.05ug
hICD 0.1ug
hICD 0.5ug
hICD 1.0ug
hICD 2.0ug
None
rICD 0.05ug
rICD 0.1ug
rICD 0.5ug
rICD 1.0ug
rICD 2.0ug
Antigens

Figure 5

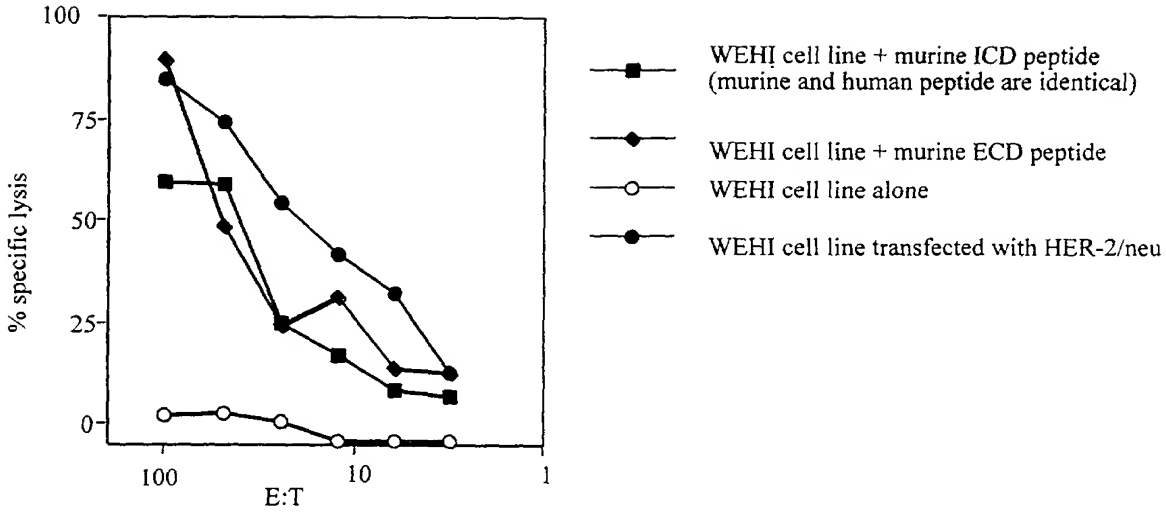

100
75
50
25
0
% specific lysis
100
10
1
E:T
WEHI cell line + murine ICD peptide
(murine and human peptide are identical)
WEHI cell line + murine ECD peptide
WEHI cell line alone
WEHI cell line transfected with HER-2/neu

Figure 6

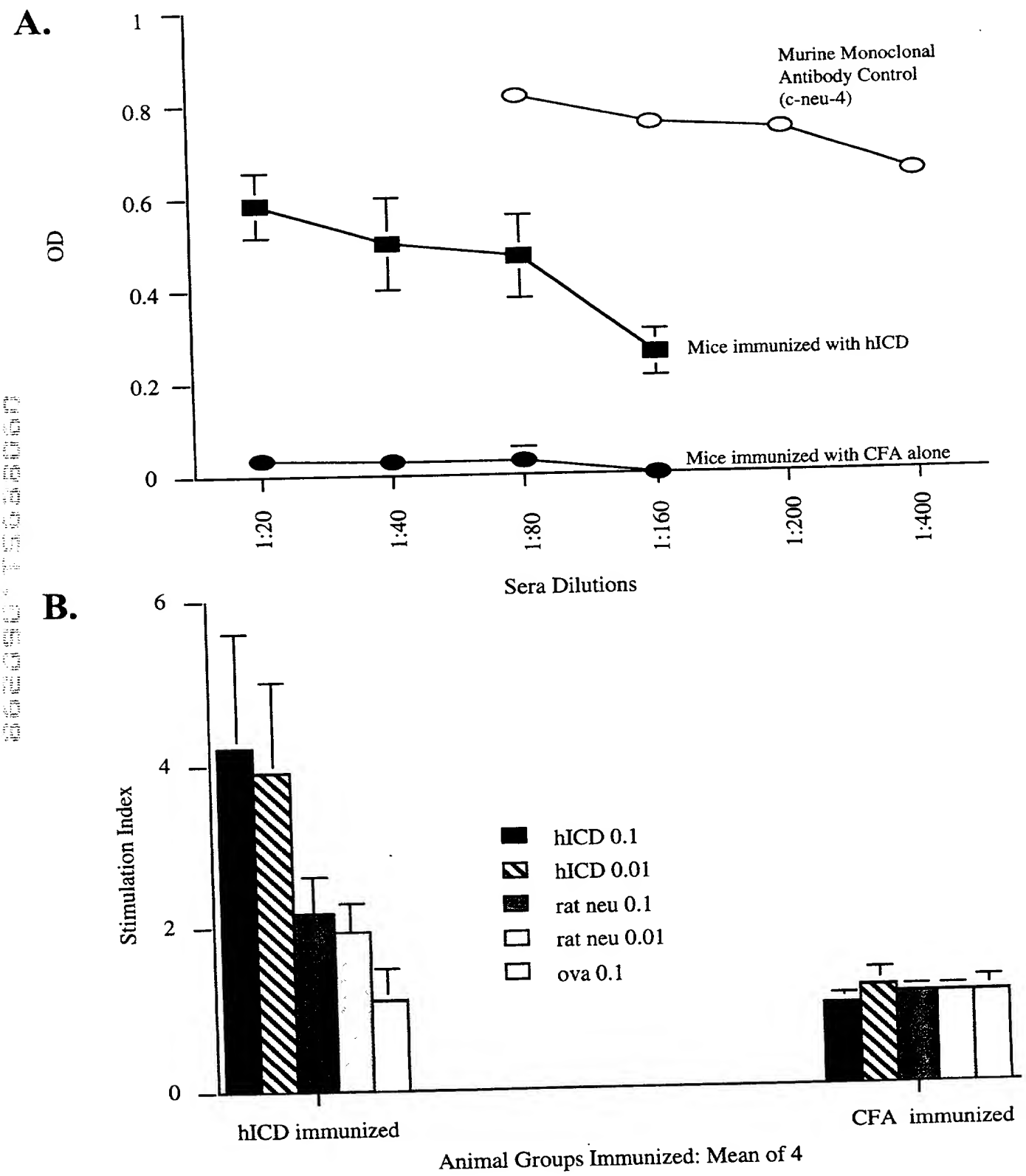

A.
1
0.8
0.6
0.4
0.2
0
OD
Murine Monoclonal
Antibody Control
(c-neu-4)
Mice immunized with hICD
Mice immunized with CFA alone
1:20
1:40
1:80
1:160
1:200
1:400
Sera Dilutions
B.
6
4
2
0
Stimulation Index
hICD 0.1
hICD 0.01
rat neu 0.1
rat neu 0.01
ova 0.1
hICD immunized
CFA immunized
Animal Groups Immunized: Mean of 4

Figure 7

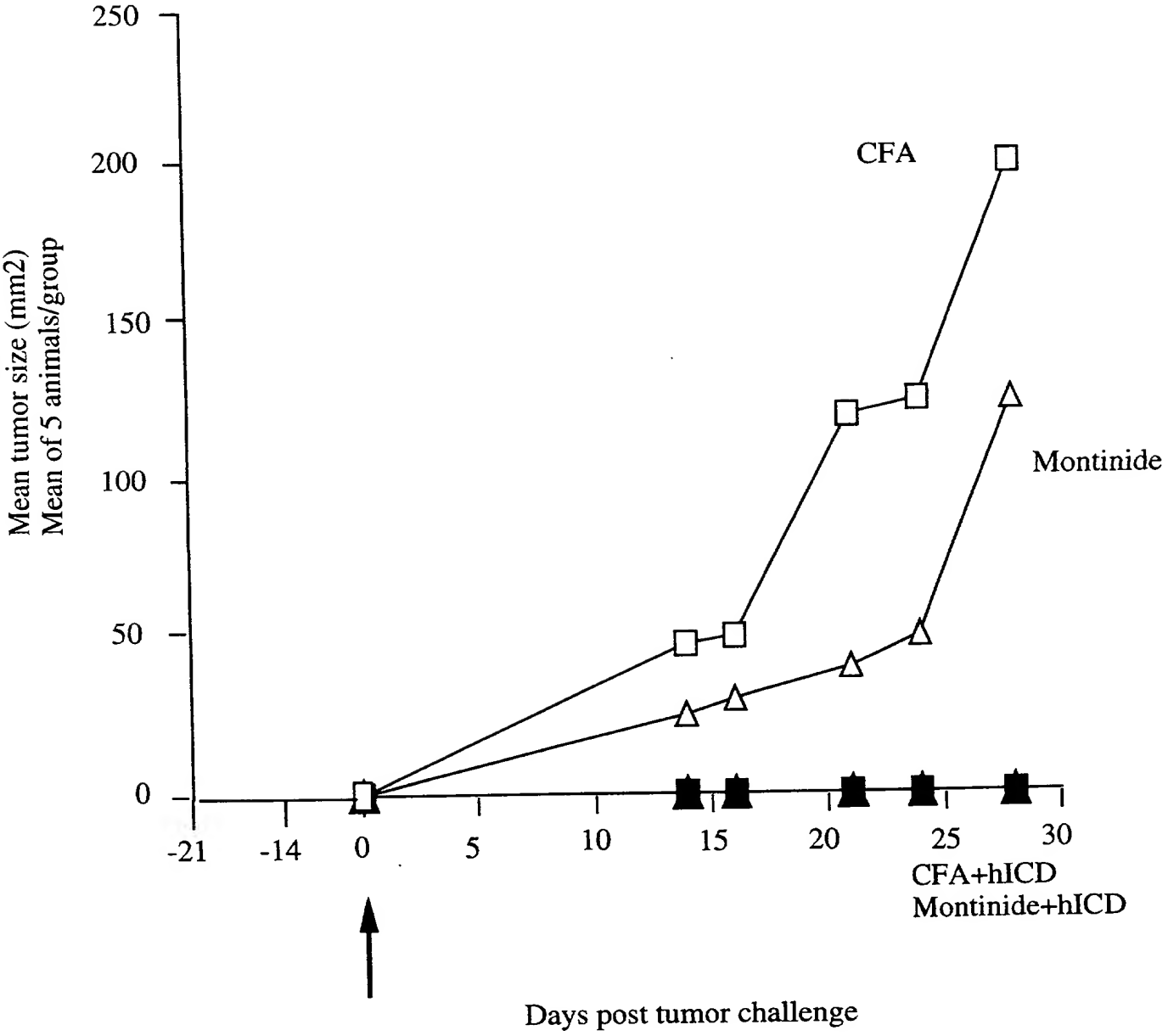

250
200
150
100
50
0
Mean tumor size (mm2)
Mean of 5 animals/group
CFA
Montinide
-21
-14
0
5
10
15
20
25
30
CFA+hICD
Montinide+hICD
Days post tumor challenge

Figure 8

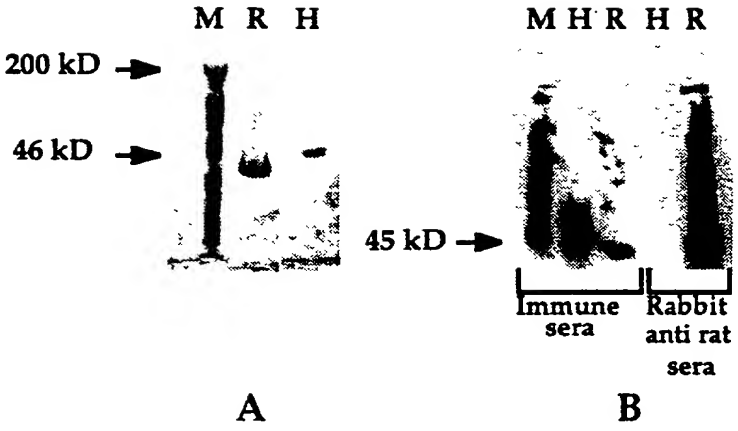
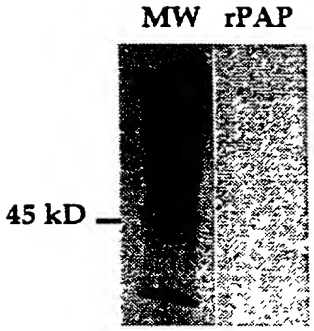
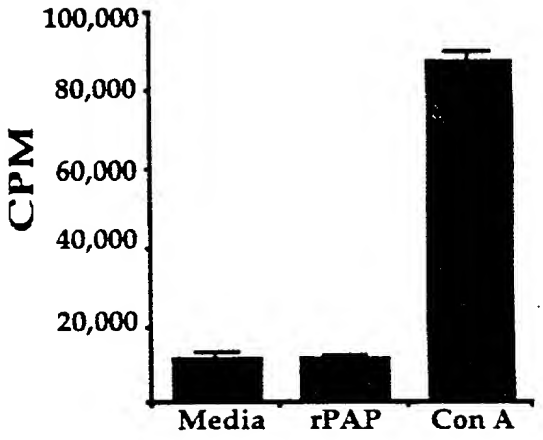

M R H
200 kD
46 kD
M H R H R
45 kD
Immune sera
Rabbit anti rat sera
A
B

Figure 9


MW rPAP
45 kD

A


100,000
80,000
60,000
40,000
20,000
CPM
Media
rPAP
Con A

B

Figure 10

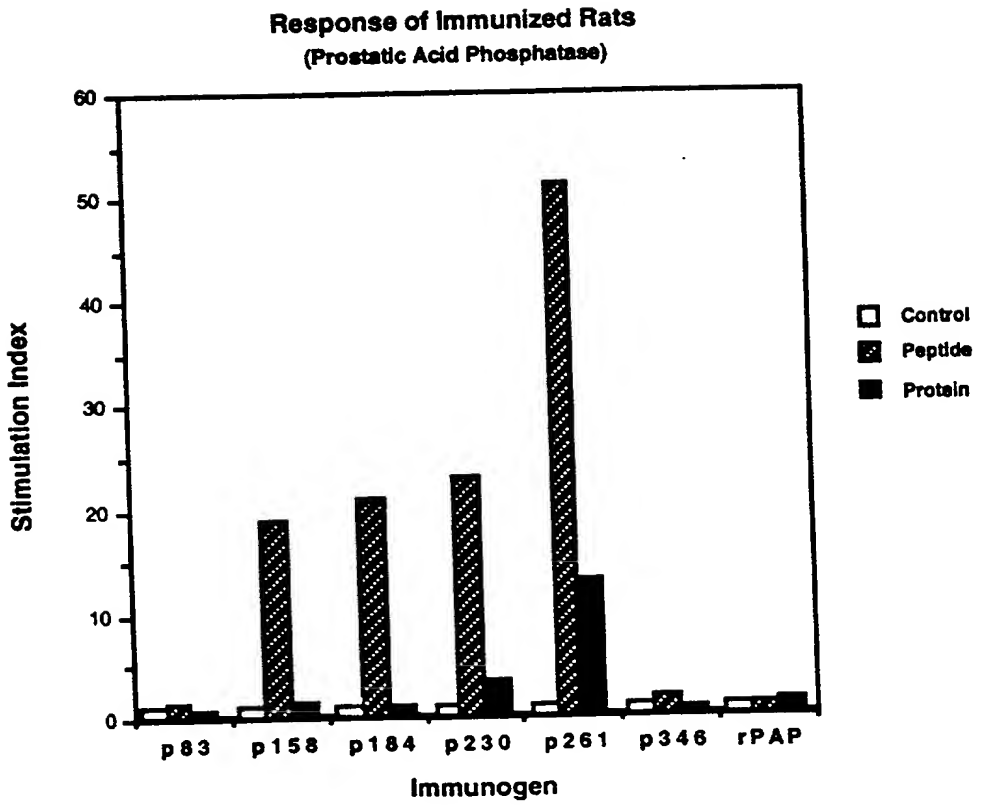

Response of Immunized Rats
(Prostatic Acid Phosphatase)
Stimulation Index
60
50
40
30
20
10
0
p83
p158
p184
p230
p261
p346
rPAP
Immunogen
Control
Peptide
Protein

Figure 11

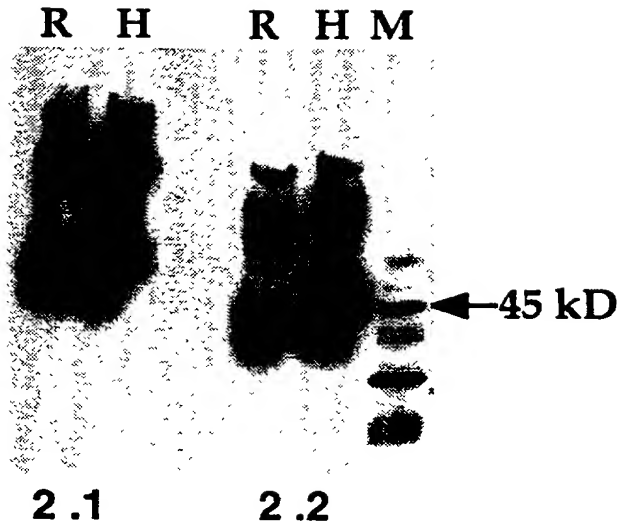


Figure 12

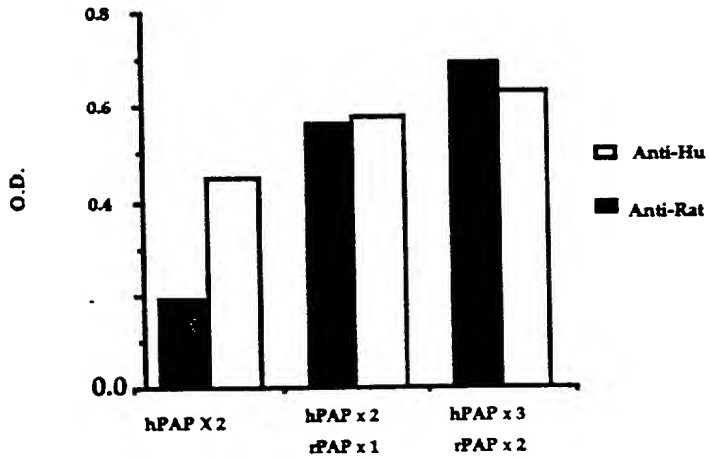

0.8
0.6
0.4
0.0
O.D.
Anti-Hu
Anti-Rat
hPAP X 2
hPAP x 2
rPAP x 1
hPAP x 3
rPAP x 2

Figure 13

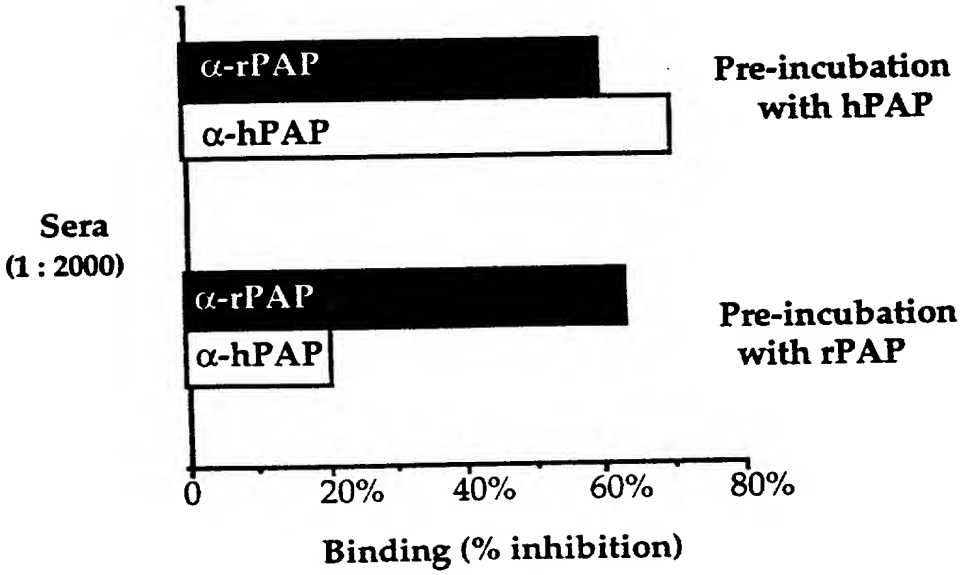

α-rPAP
α-hPAP
Pre-incubation with hPAP
Sera (1 : 2000)
α-rPAP
α-hPAP
Pre-incubation with rPAP
0
20%
40%
60%
80%
Binding (% inhibition)

Figure 14

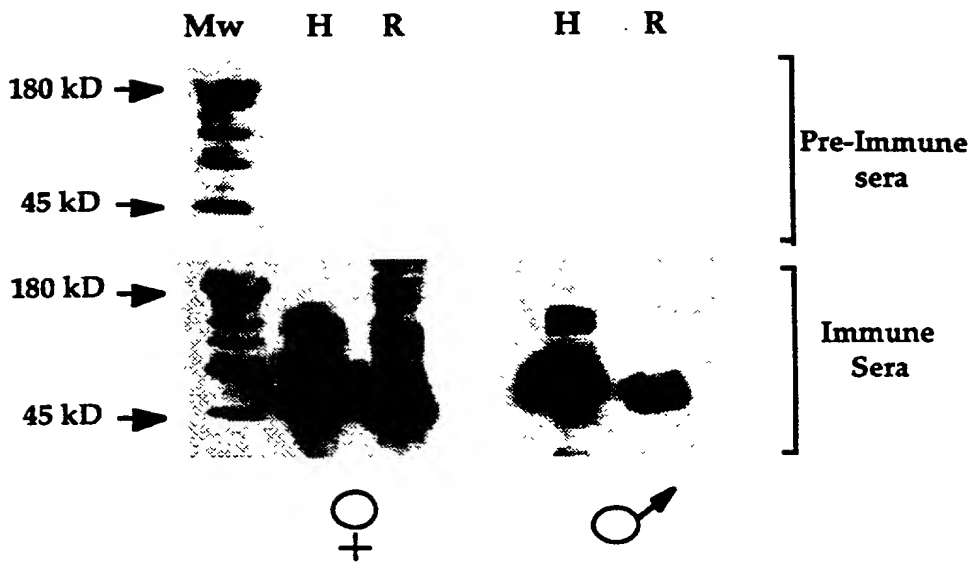


Figure 15A

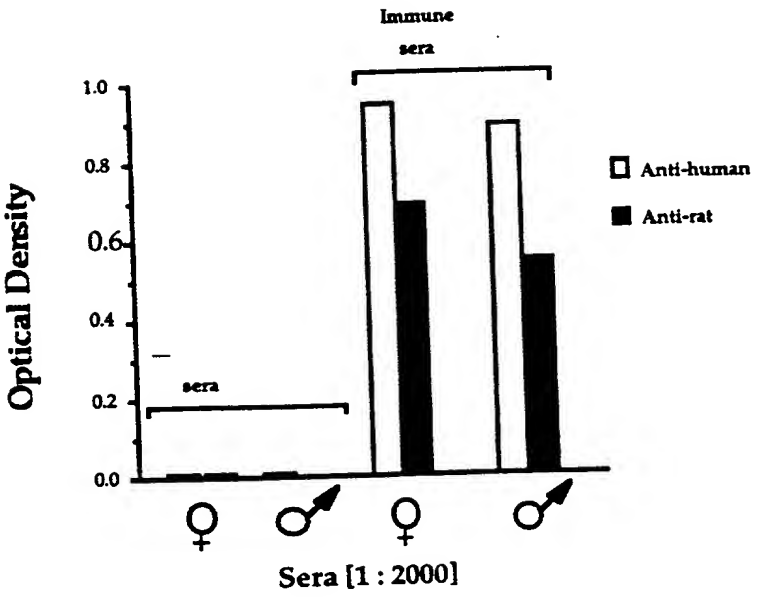

Immune
sera
1.0
0.8
0.6
0.4
0.2
0.0
Optical Density
sera
Anti-human
Anti-rat
Sera [1 : 2000]

Figure 15B

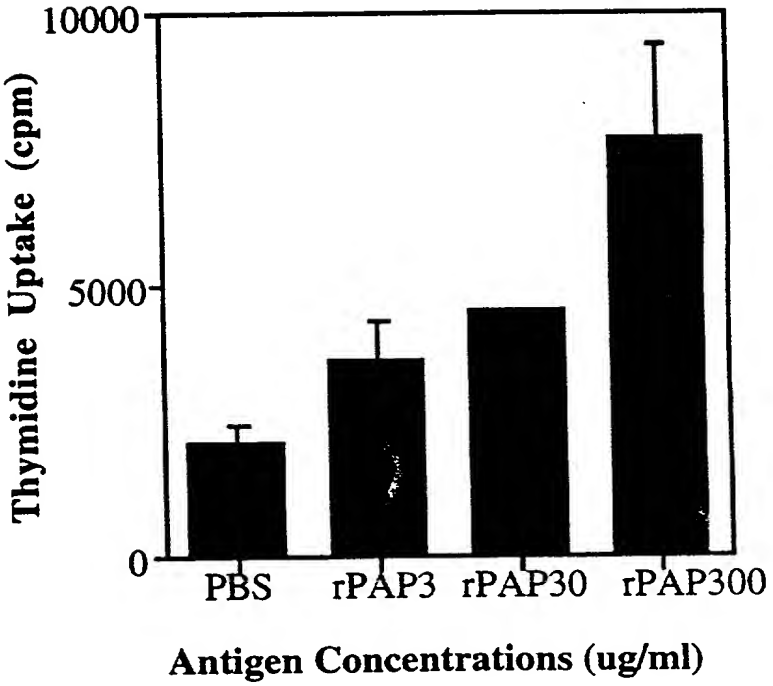

10000
5000
0
Thymidine Uptake (cpm)
PBS
rPAP3
rPAP30
rPAP300
Antigen Concentrations (ug/ml)

Figure 16

United States Patent & Trademark Office

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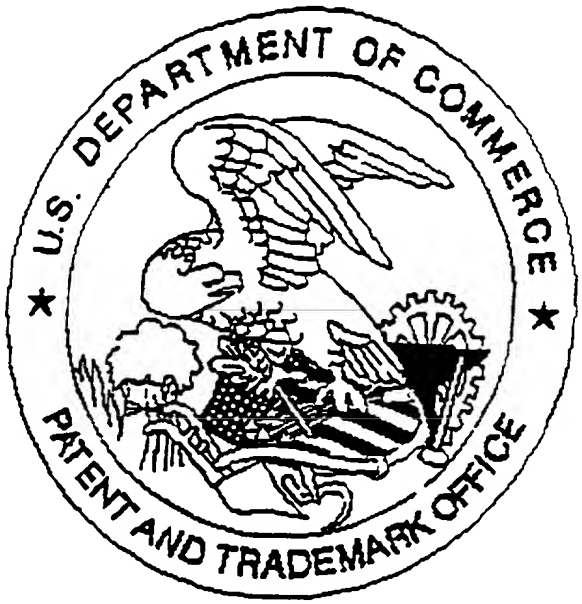

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